| K. K. Fox | Casein and Whey Protein |

CASEIN

Most, if not all, casein used in the United States is imported. Approximately 100 million pounds of casein were imported in 1966 and 1967 (Table 11.1). It is imported largely as isoelectric casein for two reasons: one, a tariff of 2.75¢ per lb is imposed on sodium caseinate; and, two, isoelectric casein has better keeping qualities than sodium caseinate. As may be seen in Table 11.2, about ⅔ of the casein is used for nonfood purposes. Its primary industrial uses are as a binder in paper, paint, and adhesive products. Reportedly, casein is being slowly replaced in these industries by other proteins, modified starch, and polyvinyl alcohol. At the same time, casein is being used in newer food products such as filled milk, imitation milk, coffee whiteners, instant breakfasts, cereals, sausages, toppings, ice cream mixes, and other foods where it is used to supplement the proteins already present.

The domestic manufacture of casein is apparently depressed by a Federally supported pricing structure for milk that encourages the production of dried milk rather than casein from milk (Anon. 1968A).

Most of the research and development work on casein manufacture and properties that has been published since 1950 has been done by the Division of Dairy Research, Commonwealth Scientific and Industrial Research Organization (CSIRO), Melbourne, Australia. The only new process that has been introduced in casein manufacture since 1950 has been coprecipitation of the whey proteins with the casein. As much as 97% of the total protein of milk can be coprecipitated. This procedure will be outlined in some detail later.

Although very little, if any, casein is produced in the United States, segments of the dairy industry have kept abreast of developments in this field as indicated by the issuance of patents covering newer manufacturing procedures. These include the procedure of coprecipitation. Also, it seems likely that some casein is again being manufactured in this country for the growing food usage which requires a fresher tasting

TABLE 11.1

IMPORTS OF CASEIN AND CASEINATES

1966

Casein	
Origin	Lb
New Zealand	26,799,000
Argentina	25,484,000
Australia	19,878,000
Canada	10,925,000
Poland	10,800,000
France	6,335,000
Other countries (27 in all)	4,109,000
Total casein	104,330,000
Caseinate	3,576,000
	107.9 million

1967

Casein	96.6 million
Caseinate	3.1 million
	99.7 million

SOURCE: McCabe (1968).

TABLE 11.2

USAGE OF IMPORTED CASEIN AND CASEINATES, 1967

	Million Lb
Food, pharmaceuticals, and pet food uses	33.0 casein
	3.1 caseinate
Paper coating and related uses	34.0 casein
Glues	10.0 casein
Plastics	1.5 casein
Miscellaneous	18.1 casein

SOURCE: McCabe (1968).

product than can perhaps always be recovered from imported casein. As used in the food industry, casein must nearly always be converted to sodium caseinate. The solution and conversion of dried casein to sodium caseinate with subsequent redrying as sodium caseinate entail extra labor and some losses of product. Such losses could be avoided if the sodium caseinate were to be made from wet, but fresh, casein curd, which also would allow the manufacturer to have control of his product from the raw milk to the finished product.

As indicated by the data in Table 11.1 almost 45% of the casein imported in 1966 and 1967 came from New Zealand and Australia. As of about 1956 New Zealand had become the world's largest producer of casein; previously Argentina had been.

Manufacturing Procedures

Precipitation.—Casein exists in milk as a calcium caseinate-calcium phosphate complex. When an acid is added to milk, this complex is dissociated. As the pH of milk is lowered, the calcium is displaced from the casein molecules by hydronium ions, H_3O^+, and the calcium phosphate associated with the complex is converted into soluble Ca^{2+} ions and $H_2PO_4^-$ ions. At about pH 5.3 the casein begins to precipitate out of solution; at the isoelectric point of casein, about pH 4.7, maximum precipitation occurs. Also, the pH must be lowered to at least this point to completely solubilize the calcium phosphate. The casein coagulates as a watery, gel-like curd. The gel is broken to allow water to be expelled from it. The expulsion of water by contraction of the curd, or syneresis, is aided by further development of acidity and by heating, or a combination of both factors.

Effect of Temperature.—The kind of curd formed is quite sensitive to heat, and if the acid is being developed through fermentation, the rate of acid development is also sensitive to temperature. Consequently the temperature during precipitation must be controlled closely within narrow limits. Curd precipitated at temperatures below 95°F is soft and fine. Precipitated between 95° and 100°F, the curd is coarse, provided stirring is not too fast. The curd can be made firm in either of two ways: by heating between 100° and 118°F, the temperature depending on the acidity; or the pH lowered to 4.1 without raising the temperature. This latter method also produces a curd that is easy to wash and drain. Curd precipitated at about 110°F has a texture resembling chewing gum, being stringy, lumpy, and coarse, containing no fine particles, and separating cleanly from the whey. When such a curd cools to below 110°F, it stiffens and can be broken easily by agitation into coarse grains. Therefore to control the process of acid precipitation in making acid casein the two variables of most concern are: (1) the pH of precipitation, and (2) the temperature of precipitation.

Draining and Washing.—The longer curd stands in contact with the whey, the more difficult it is to wash out acids, salts, whey protein, and lactose. The freshly broken curd tends to anneal itself, thereby enclosing these constituents within a protein film. Consequently, it is important that the whey be removed from contact with the curd as soon as possible after precipitation has been completed and that the curd be washed immediately. Inadequately washed casein is less soluble than thoroughly washed casein, has less adhesive strength, and develops more color on drying.

The pH of the wash water should be the same as that of the casein, so as

to be in equilibrium with it. With an overacid water a gelatinous layer may be formed over the curd particles, which inhibits drainage of salts and lactose from the particles; alkaline water will tend to redisperse and soften the curd. In addition to the proper pH, the wash water must be free of colors and turbidity, as these are readily absorbed by the casein and will affect the color of the finished product.

Muller (1959) found that lactose, salts, and acid separate from the curd particles by the process of diffusion during the washing process. Consequently the factors which affect the diffusion rate are of importance in the washing process. The diffusion rate depends on the size and permeability of the curd particles, and the purity, amount, and rate of movement of the wash water. Muller found that to obtain efficient washing, 3 separate washes of the casein curd were required with contact times of 15–20 min each. In a continuous system, a countercurrent flow of water was found to use a minimum amount of water, and also by this arrangement the loss of fines in the wash water occurs only once.

When the casein is to be used for food products, it is necessary to use a hot wash water of 160°–170° F at some stage. This effects a pasteurization of the curd for the purpose of reducing the number of bacteria present. It has also been found that for efficient pressing of the curd in the continuous-type press, the final wash water should be about 105° F.

According to Neff (1966) the continuous washing process consists of moving the curd through a series of vats. The top of each vat is fitted with an inclined screen which separates the incoming curd from the whey or wash water. The incoming curd and wash water are agitated together, and the agitated mixture is continually pumped from the bottom of the vat to the next vat. Fresh water may be introduced at each vat or only one lot of fresh water introduced at the last vat, and this water reused in countercurrent flow. A modified countercurrent flow can also be used in which 2 fresh water washes are added at the last 2 vats and these 2 wash waters reused in a "weaving" flow between vats.

Pressing.—The amount of water in washed curd and its ease of removal depend upon the type of curd made. The percentage of water can be reduced by pressing to not lower than about 55%. In batch pressing, the washed and drained curd is shoveled into press cloths supported in a form. When all the curd is in the press cloths and on the press, the press is tightened, only moderately at first, but with increasing pressure as more of the free water is pressed from the casein curd. The pressing operation is usually an overnight operation, being started in the late afternoon and completed the following morning.

Curd can also be pressed continuously in mechanically-driven roller presses where one press is sufficient to obtain the expulsion of water necessary for subsequent economical drying. However the curd is pressed,

its final moisture content depends largely on its initial condition. Precipitation of the curd at a pH of 4.1–4.3, and the curd well-washed in waters, also of the proper pH, and at temperatures of 105° F will give a firm, friable curd which will drain well and press well.

Milling and Drying.—After pressing, the curd is milled to produce particles of a uniform size and surface for drying. Otherwise, uneven drying occurs. Large particles or lumps may dry on the outside forming a hard, impervious outer surface that prevents the diffusion of the remaining moisture from the interior of the particle. Instead of grinding, the broken curd may be forced through perforations onto drying trays or belts. The kind of grinder used is dictated by the kind of drier used. An early detailed account of drying equipment is by Spellacy (1953). Buchanan *et al.* (1967) and Neff (1966) provide a more recent discussion of driers in use in New Zealand and Australia. The present type of drier being installed in these areas is a vibratory one referred to as the Bates Drier. The Bates Drier is made in 2 sections: a predrier with 2 trays mounted one above the other in which the moisture content is reduced to 30–35%, and a final drier consisting of four trays similarly mounted. The milled curd travels by a vibratory motion which ensures uniform movement of the curd throughout the drying process. This avoids scorched and burnt particles. The curd, after traveling the full length of each tray, transfers to the next lower deck by means of star-valve control (rotary air-lock). A current of hot air is applied to the lower chamber in each section and passes countercurrently up through the curd located on the various screens so that some fluidization of the casein occurs. The partially dried curd is transferred from the predrier to the final drier pneumatically, i.e., by means of an air stream. Separation of the properly dried particles from incompletely dried particles is made finally by means of a cyclone. Advantages listed for this type of drier are: elimination of overdrying of part of the casein, high efficiency of heat utilization, and the flexibility to cope with varying types of curd. Also the predrier can be used efficiently with other kinds of driers. This drier can handle curd from a maximum of about 4000 gal. milk per hour.

The Sheffield continuous air-suspension drier has been described by Spellacy (1953). In this process the curd is carried in air streams through two drying chambers constructed similarly to cyclone air-separators. Cesul *et al.* (1963) describe a continuous drier of the fluidized-bed type, consisting of a vertical cylinder with the dry product being expelled from the top. The principle of fluidization and some applications including drying are well-discussed by Flood and Lee (1968).

Tempering.—Tempering means the holding of the casein for a period (24 hr) to allow efficient cooling, hardening of the casein, and evenness of moisture throughout the batch. Casein shows variation in moisture content

during a day's run as it comes from the drier. Agitation is necessary for efficient tempering. According to Neff (1966) the most efficient tempering consists of recirculating the dried casein by pneumatic conveyance. It has the advantage that air used for transport of the casein assists in cooling the curd. The dried casein is withdrawn from the bottom of a bin and pneumatically conveyed into another bin or recirculated back into the same bin.

Grinding.—The cool, tempered casein is ground to the desired particle size. The casein must be cool before grinding because warm casein is plastic and causes "burn on" of the rollers. The grinding is done by roller mills as in the flour mill industry.

Sieving.—After grinding, the casein is sifted into its various mesh sizes. Common mesh sizes are 30–40 mesh casein, 60 mesh casein, and 90 mesh casein. An object of the grinding and sieving operations is to produce the highest proportion of the product in the size range desired by the buyer. The largest market is for casein in the 30–40 mesh range. However some market exists for all other sizes.

Bagging.—After sieving, the product is bagged according to mesh size. The kind of bag used may be prescribed by the grade classification of the casein.

Types of Casein

Casein may be typed according to the process used to precipitate it as follows: (1) hydrochloric acid casein, (2) sulfuric acid casein, (3) lactic acid casein, (4) coprecipitated casein, (5) rennet casein, and (6) low viscosity casein.

Hydrochloric Acid Casein.—When casein is precipitated from skim milk by the direct addition of acid, the temperature and pH of precipitation, and the mechanical handling of the curd during its formation were found by Muller and Hayes (1962) to be very important in determining the subsequent properties of the curd. Since the problems that had to be overcome are informative they are presented here as well as the manner of their resolution. For efficient mixing, the acid and the milk were mixed inside of a 2-in. pipe. However, if the milk was too warm (110°–115°F), curd formed within 1/10 sec and the shearing force on the still-forming curd being expelled from the mixing tube imparted undesirable qualities to the curd. Above pH 4.4 the resulting curd was fibrous and sticky and wound around agitators and blocked pumps. A second kind of defect occurred when the impeller of a pump was used to mix the acid with the skim milk. At temperatures of 110°–115°F the coagulation occurred so rapidly that the curd was damaged by the pump impeller as it was being formed. The solution of the problem consisted of acidifying the milk at

86°–90° F, then immediately warming the milk to 110°–115° F by steam injection. Also included was redesign of the mixing equipment. A mixer was designed to inject the acid countercurrently into the milk line in the form of a cone-shaped spray with sufficient force to produce turbulent mixing. Both the milk and the acid were moved by positive action pumps so as to ensure a constant ratio by volume of acid to milk. The acidified and warmed milk (now 110°–115° F) flowed onto a primary chute and then to a riffle chute where coagulation occurred. The size of the aggregates formed was controlled by adjusting the slope of the riffle chute. Minimum losses were obtained by employing pH values of 4.25–4.35. The acid was applied at a concentration of 1.3 N–1.4 N. Either hydrochloric or sulfuric acids can be used. No drift in pH occurred when the curd and whey were held together for 30 min. This indicated that immediate and complete action of the acid had been obtained. As a consequence, the whey could be drained and wash water of the proper temperature substituted without the use of an acidulation vat. The latter is the vat or pipeline described in the lactic acid procedure for maintaining a temperature and time of heating for regulating curd formation.

Sulfuric Acid Casein.—Sulfuric acid casein can be manufactured by employing a process identical with that for hydrochloric acid casein. Also an equally high quality product can be produced by the use of sulfuric acid (King *et al.* 1962). There is basically no difference between any of the acid caseins. Prejudice against sulfuric acid casein exists because in the past the term was associated with a low quality cooked-curd casein. A reason for using sulfuric acid is that it costs much less than hydrochloric acid. Neff (1966) reports the costs per ton of casein as $5.50 for sulfuric acid as compared to $24 for hydrochloric acid. A possible drawback to the use of sulfuric acid is that the whey cannot be used for animal feed. McDowall *et al.* (1962) found that pigs fed sulfuric acid casein whey showed abnormalities of the carcass and intestines.

Lactic Acid Casein.—According to Newport (1967) the production of casein by the souring of skim milk by means of cultures of lactic streptococci is preferred for economic reasons by most manufacturing companies in New Zealand. The skim milk is held in vertical storage tanks of up to 20,000 gal. capacity for approximately 16–18 hr to coagulate. The skim milk is inoculated with 1/2% starter at 80° F. The starter is of mixed strain and is transferred daily. The clotted skim milk is transferred from the tank with a special type pump, and at the same time the curds and whey are heated by a direct steam injection. The temperature to which the milk is heated will vary according to the nature of the coagulum but will be in the range of 120°–140° F. With lactic acid casein an acidulation time is necessary. This is a time period during which the milk, or all the milk

reaches the pH of precipitation and the calcium salts are solubilized and dissociated from the casein. After the curd has formed, the acid and heat cause shrinkage of the curd (syneresis) with moisture expulsion. The acidulation period is regulated by either an acidulation vat or a holding tube such as a longer and/or larger section of pipe.

From the acidulation vat or pipeline the curd flows onto an inclined dewheying screen. The inclination of the screen is such that the curd falls by gravity into the first washing vat. The curd is washed in multiple stages at temperatures to eliminate lactose and soluble salts and at the same time maintain desirable curd characteristics.

Rennet Casein.—The enzymes rennin and pepsin displace a glyco-macropeptide from the κ-casein fraction of casein. In the presence of calcium ions, the casein, hereafter referred to as rennet casein, precipitates. Rennet casein is a product different from acid casein and industrially is used for different purposes. The skim milk used for manufacture of rennet casein must be of high quality since milk of poor quality with developed acidity will have some of its calcium solubilized which will result in a poor curd and should be avoided. Sufficient rennet and calcium chloride are added to the skim milk to give a setting time of 20–30 min. Agitation of the coagulum is begun 2-5 min after coagulation begins, but before the coagulum reaches a solid clot, to give an optimum size curd for further processing. As soon as agitation of the curd is begun, the temperature of the curd is raised to 130°–150° F. The curd is cooked at this temperature for about 30 min. After cooking, the curd is processed in the same manner as acid caseins. Rennet casein is used in making casein plastics.

Coprecipitates.—Scott (1952) and Howard et al. (1954) describe batch methods of making a coprecipitate in which acid was the precipitating agent. No provision was made for controlling the calcium concentration. Calcium has since become a desirable component in the coprecipitate. A possible method for the continuous production of a calcium coprecipitate of casein and whey proteins from heated milk by calcium chloride was described by D'yachenko (1957). Such a continuous process developed in Russia was described by Arbatskaya et al. (1962). Research and development leading to a manufacturing procedure for calcium coprecipitates are described by Buchanan et al. (1965), Muller et al. (1967), and Smith and Snow (1968), all of the Division of Dairy Research, CSIRO, Melbourne. Muller et al. (1967) report on an investigation at CSIRO undertaken to develop continuous processes for the production of coprecipitates with calcium contents adjustable over a wide range. They concluded from their findings the following. (1) Two-stage heating of the skim milk should be used because it allows heat to be recovered from the hot whey which

could be used to preheat the milk. (2) A final temperature of 195° F was optimum because protein recoveries increased with temperature up to 194° F. It was also found that about 190° F was the minimum final temperature, if a firm, easily processed curd was to be obtained. The final temperature of 195° F was obtained by steam injection into the milk in a holding vat. (3) The time of holding of the milk at 195° F was regulated by balancing the rate of flow into and from the holding vat. (4) After the holding stage, calcium chloride solution or acid was injected through a spray countercurrent to the direction of milk flow to give good mixing. (5) The mixture moved through a pipeline of such dimensions as to give 20–25 sec holding time before discharge onto a riffle chute. (6) Separation of curd from whey on an inclined screen is followed by a continuous washing process in a series of vats. The washing conditions can be varied to adjust the composition of the final product. (7) Pressing and drying of the curd follow after washing.

The curd formed by coprecipitation of casein and whey protein has physical characteristics different from those of a casein curd. The authors cite as an example that, if casein is precipitated at 110°–120° F at a pH above 4.6, the curd will be fibrous and sticky. A coprecipitated curd at even higher temperatures and a wide range of pH is seldom fibrous or sticky. The authors suggest that the difference in behavior of the curd of a coprecipitate from a regular casein curd is due to the interaction of the casein with β-lactoglobulin on heating. A recovery of about 96% of the milk proteins was achieved under optimum processing conditions. The level of calcium in the coprecipitate was determined by the pH of precipitation, the amount of calcium chloride added, and the washing conditions.

A low-calcium product is considered to contain about 0.5–0.8% calcium and is obtained by adding calcium chloride equivalent to 0.03% of the weight of skim milk before preheating at 155° F followed by heating to 195° F and holding at this temperature for 15-20 min. Precipitation follows by acid addition to pH 4.6.

A medium-calcium product (about 1.5% calcium) can be made by adding calcium chloride equivalent to 0.06% of the weight of the skim milk before preheating, followed by 2-stage heating to 195° F. After a holding period of 10-12 min, the proteins are coprecipitated at a pH of about 5.3.

A high-calcium product (about 2.5–3.0% calcium) is obtained by holding the milk heated to 195° F for 1-2 min, then adding calcium chloride equivalent to about 0.2% the weight of the skim milk. This is followed by a 20-25 sec holding time interval in a holding tube. The addition of this amount of calcium chloride suffices to precipitate the proteins without the use of any acid to lower the pH.

Buchanan *et al.* (1965) studied extensively the problems of manufacturing a high-calcium coprecipitate. To obtain maximum yields it was found necessary to avoid undue agitation during separation of the curd from the whey. Wash-water temperatures of 84°–96°F were found satisfactory. The composition was affected particularly by the amount of washing the curd received. The high fat content of the coprecipitate is attributed to retention in the curd of fat-protein complexes formed during the manufacturing process.

Smith and Snow (1968) describe the solubility properties of the three types of coprecipitate. Solubility experiments with low-calcium coprecipitate showed that its solubility properties were similar to those of casein, i.e., adjustment of the pH sufficed to put it into solution. For medium- and high-calcium coprecipitates, a calcium-sequestering agent is necessary for maximum solubility at pH 7.0. Medium-calcium coprecipitates are solubilized by inclusion of 2-4% sodium tripolyphosphate at pH 7.0. For a high-calcium coprecipitate inclusion of 6% sodium tripolyphosphate resulted in 85% solubility at pH 7.0. The solubility increased to 92–95% at pH 8.0.

Some insoluble, aggregated whey proteins are found in solutions of calcium coprecipitates at pH 7.0. To produce sediment-free solutions of the calcium coprecipitates, homogenizing was found to be necessary. How the calcium coprecipitates are dried also affects their solubility. Coprecipitates dried in a casein drier are more difficult to put into solution than the spray-dried form. Coprecipitates dried in a casein drier are referred to as a granular form. The average composition of a coprecipitate is shown in Table 11.3 (Buchanan *et al.* 1965).

Low Viscosity Casein.—The major industrial use for acid casein is in the paper industry, where it is used as an adhesive to bind pigments to paper. A technological change in the paper industry has been toward including more solids in the coating (Muller 1961). Because the viscosity

TABLE 11.3

AVERAGE COMPOSITION FOR
COPRECIPITATE[1]

	%
Protein ($N \times 6.38$)	83.0
Lactose	1.0
Fat	1.5
Moisture	4.0
Ash (including Ca)	10.5
Calcium	2.5

SOURCE: Buchanan *et al.* (1965).
[1]Spray-dried powder.

of caseinate solutions changes logarithmically with change in concentration, there has been a trend away from casein to other adhesives in the paper industry. To counter this trend Muller and Hayes (1963) investigated ways of modifying the casein to obtain a lower viscosity. They found that the action of proteolytic enzymes produced a casein which had a much lower viscosity (3-4 poise for a 15% solution instead of 20 poise). They developed a procedure on a continuous basis for the manufacture of a low viscosity casein which utilized the action of certain proteolytic enzymes on casein. So much of their procedure as is done with the enzyme pepsin is summarized here. About 40% of the milk is subjected to direct enzyme treatment. The pH of this fraction is adjusted to 3.5-4.0 and its temperature is adjusted to 106°-108° F. Pepsin equivalent to 0.05% of the casein is added. Three tanks in series provide a holding stage of 45-60 min. After the holding stage the pepsin-treated milk is blended with the remaining milk by being pumped into it in the form of a spray as it is conveyed by pipeline to a riffle chute. The pipe is of such size that the blended milk is held up about 15 sec. The initial pH of the blend should be about 5.0. After the holding treatment, acid is injected into the pipeline to lower the pH to 4.6; steam is injected to raise the temperature to 120° F. Sufficient holdup is made with the riffle chute for curd formation to occur. To obtain a product low in calcium and ash, the wash water is adjusted to pH 4.2-4.3. Aside from its increased utility in the paper industry, low viscosity casein could reduce costs of drying caseinates and could expand the use of casein for food products.

Sodium Caseinate

The problems of the manufacture of sodium caseinate have been well discussed by Burston et al. (1967). It is estimated that 10-15% of the world's production of casein is used for food. A large proportion of this would be as sodium or calcium caseinate. Caseinates for food use may be made either from wet, freshly-precipitated casein curd, or from edible grades of dry casein. The manufacturing procedure consists of preparing an aqueous colloidal suspension from acid casein at pH 6.7 by addition of alkali, usually sodium hydroxide. The solution is then dried, commonly by spray drying. It is pointed out by Burston et al. (1967) that the basic problem of the process is the relatively high viscosities of sodium caseinate solutions at moderate concentrations. Factors involved in the control of viscosity are concentration, pH, and temperature. At their optimum, the concentration of sodium caseinate that can be handled is 20-25% at a temperature of 194°-200° F, and a pH of 6.2-6.7. As a consequence the output of a spray drier drying caseinate is about ¼ that of drying a milk

concentrate of 40-45% total solids. The cost of drying caseinates is proportionately higher than that of milk solids.

Starting Material.—As would be expected, the best flavor in dried sodium caseinate is obtained in a product manufactured from fresh wet curd. Calcium is a cause of variations in viscosity and a curd of uniformly low calcium content is desired. The lactose content should be low since this sugar reacts with the casein during drying to cause discoloration of the product. Also through the same reaction, i.e., between lactose and the ε-amino groups of lysine, the nutritional value of the product is decreased. To minimize viscosity and total solids variation in the caseinate solution, the curd should have a uniformly low moisture content, the latter being controlled by conditions of precipitation, washing, and pressing.

Dissolving Technique.—Well-made wet curd contains 55-60% moisture. Consequently 100 lb of a 20% solution would entail the addition of 50 lb of water and alkali to 50 lb of a 60% moisture curd. According to Burston *et al.* (1967) experiments show that the viscosity at a given temperature during the mixing process depends on the number of undispersed particles in the mix at that stage of mixing. As a result, if it is attempted to make a physical mixture of 20% casein (the curd finely divided) and then add alkali to the mixture, a very high viscosity is attained. A second problem with wet curd which may be responsible for the above effect is that the particles in the presence of alkali form a viscous coating on the surface which resists further dissolving action. These two problems are resolved by adding the wet curd and alkali into a vat equipped with a powerful agitator. A large capacity centrifugal pump is attached to the outlet of the vat and used to recirculate the contents. The alkali is added to the vat on the suction side of this pump so that vigorous mixing action within the pump prevents formation of the heavy viscous surface on the curd particles. By this arrangement it is reported that wet curd and alkali can be progressively added without undue difficulty. However, as the concentration and therefore the viscosity increases, this equipment arrangement can no longer dissolve the curd. To obtain a final solution concentration of 20% solids it is necessary to pass the mixture through a colloid mill. Finally, sodium caseinate solutions before spray drying must be standardized for viscosity rather than concentration. However, when uniform quantities of casein are being processed, knowledge of the total solids is also useful. This may be determined by a hydrometer procedure, by a refractometer, or by a gravimetric process. The relationship between the refractive index of sodium caseinate solutions and their concentration is linear between 15 and 26% and is repre-

sented by the equation;

$$\%ts = 490.67R - 651.67$$

where R is the refractive index at 104°F (Anon. 1966).

Temperature Control.—According to Burston *et al.* (1967) there are two limiting factors in the use of high temperatures in sodium caseinate manufacture. The first is the tendency for curd particles to toughen in hot water and become even harder to dissolve in alkali. The second is the effect of high temperatures on the quality of the dried caseinate. The first factor can be overcome by the use of a powerful recirculating pump, a powerful agitator in the mixing vat, followed finally by putting the solution through a colloid mill.

The second factor is related to the amount of lactose present. Above 158°F, caseinate solutions on prolonged exposure to such temperatures show discoloration unless the solution is virtually free of lactose. When caseinate solutions are to be held some time before drying it is recommended that they be held at temperatures below 158°F. Upon passage to the spray drier, heating to 195°F is necessary to ensure minimum viscosity at the spray nozzle.

pH Control.—The pH-viscosity curve for sodium caseinate is at a minimum in the pH range 6.6–7.0 (Hayes and Muller 1961). Consequently prudence suggests staying within this range. Burston *et al.* (1967) suggest that it is better to stay below the final desired pH and adjust up to it by addition of alkali rather than down to it by addition of acid. Addition of acid causes localized precipitation which is undesirable. The above authors state that a good pH meter is necessary in the processing room. It should be equipped with a temperature compensator, a glass electrode, and a flushing-type calomel electrode. With this type of electrode a drop of saturated KCl can be run through a capillary tube to give a fresh surface for each determination. The viscous, sticky sodium caseinate solutions readily cause blocking of the normal sintered-glass, calomel electrode surface.

It was stated that the curd used as a starting material should have a low calcium content. The effect of pH of precipitation on the calcium content of casein is shown in Table 11.4. Hayes and Muller (1961) stated the sharp increase in the amount of calcium retained above a precipitating pH of about 4.6 was evident in caseins precipitated with either lactic acid or hydrochloric acid. The relationship shown in the table was not exact, probably due to differences in size and consistency of curd particles making up the various caseins. Calcium removal from large, fibrous curd particles would

TABLE 11.4

THE CALCIUM CONTENT OF CASEIN AS
RELATED TO THE pH OF PRECIPITATION

pH of Precipitation	Calcium Mg/Gm
3.9	0.05
4.15	0.25
4.45	0.90
4.65	3.95
4.85	9.2
5.1	9.7

Source: Sawyer and Hayes (1961).

not be as efficient as from smaller, granular curd particles. Precipitation of casein with sulfuric acid gave caseins of lower calcium content, an effect associated with the insolubility of calcium sulfate.

Genetic Variation.—In a study on the seasonal variation in the viscosity index of casein, Southward and Dolby (1968) found that casein from one cow (from an experimental herd of six) had a consistently low viscosity index. This was shown to be due to a rare genetic variant of α-casein. The viscosity index of casein from this cow was approximately $\frac{1}{3}$ the value of the index of the casein from the other cows. Casein from the cow's identical twin had an equally low viscosity index. Electrophoretic examination showed the presence in these two low-viscosity caseins of the α_{s1}-A genetic variant present in a bloodline of Holstein cows (Thompson et al. 1962). Confirmation of the presence of this genetic variant in this instance was made by Dr. M. P. Thompson, EURDD, U.S. Dept. of Agr. Southward and Dolby (1968) suggest that the α_{s1}-A casein has a lower viscosity presumably through forming smaller aggregates in solution. Another possible explanation is that α_{s1}-A casein is not nearly as highly hydrated at low temperatures (104°F) as the more common genetic types of casein.

Sadler et al. (1968) studied skim milks differing in α_{s1}-casein type for toughness of curd produced by rennet. The toughness of curd was measured as the grams of force necessary to make penetration of the curd. At pH 4.8, milks containing α_{s1}-A casein produced very much softer curd than α_{s1}-casein types AB, B, or BC. Conceivably, milk from a herd many of whose members gave milk containing the α_{s1}-A casein could cause problems of a very serious nature.

Viscosity Control.—Since it is necessary to standardize the caseinate solution to a given viscosity rather than to a concentration before drying, it is necessary to have a viscometer in the control laboratory. It is essential that it be equipped with facilities for temperature control. A

particular spray drier may work best at a viscosity equivalent to a lower value than 20% concentration, or possibly a higher concentration may be handled. Therefore, it is necessary to determine the relationship of readings of a particular viscometer at a given temperature with the performance of a given spray drier.

Uses of Casein

The major uses of casein are: (1) paper coating, (2) adhesives, (3) foods, (4) plastics, and (5) man-made fibers.

Paper Coating.—Mineral pigments in a thin coating are applied to paper to produce a smooth, glazed surface. The pigments may be clay, blanc fixé, or titanium oxide. To make the pigment particles adhere to the paper and to one another, an adhesive is required. Casein is a very satisfactory adhesive for this purpose. In addition to binding the pigment to the paper, casein also improves the printing surface and may waterproof it to any extent desired. This latter property is essential in preventing sheets of paper in close contact from sticking together and in making wallpaper and playing cards waterproof.

Muller (1961) investigated the markets for Australian casein and found that the major use of casein was for paper coating. He estimated that more than 70% of acid casein was used for paper coating. The major markets in 1961 were in the United States and Japan. No similar study is available for casein manufactured in South America. Loss of some markets to soya bean protein due to higher price and poorer quality of casein forced attention to be given to the quality and consistency of the casein produced. As a consequence, in Australia casein is no longer considered as merely a byproduct of the dairy industry necessitating little care in its manufacture. According to Neff (1966), new installations are being constructed of stainless steel and are capable of producing the highest quality casein.

For paper coating the main quality requirements are the following. (1) Freedom from insoluble particles, which cause specks on paper. (2) Maximum adhesive strength. The usual causes of poor strength are a high percentage of ash or other impurities. Also, decomposition of the casein through the action of molds or bacteria decreases its adhesive strength. (3) Low viscosity, to facilitate application of the coating in high speed machinery. Calcium in the casein is the cause of high viscosity. (4) Minimum fat content. The "fish-eyes" are usually attributed to an excess of fat in the casein. (5) Good color. The brown color of burnt casein is imparted to the surface on which it is coated. (6) Uniform quality. Uniform quality

from batch to batch avoids the time-consuming adjustment of machinery and the changing of the composition of the coating mix.

Other Industrial Uses of Casein.—As indicated by Table 11.2, 29.6 million lb of casein were utilized under the headings glue, plastic, and miscellaneous. The use of casein has been discussed by Sutermeister and Browne (1939), and by Salzberg (1965). Although casein has been partially replaced by synthetic polymers for the manufacture of some industrial items such as plastics and paints, it seems not to have been totally usurped in any field, including that of leather-finishing. The development of a protein/synthetic fiber composite that is a silk substitute has been announced by the Toyobo Co. of Japan (Anon. 1968B). Called K-6, it is reportedly 30% casein and 70% acrylonitrile. It is claimed that this composite fiber is closer to natural silk in appearance, hand, and other physical properties than two other recently introduced silk substitutes. The company plans to start a metric-ton-per-day production unit in Sept. 1969. This represents a market for approximately 200,000 lb of casein or its equivalent of over 6 million pounds of milk annually.

In general the manufacture of industrial products is quite technical and information on methods is not available outside the industry.

Quality Control by Viscosity.—In products containing casein such as adhesives or paper coating, viscosity is important in their preparation and application. Consequently the viscosity of a sample from a batch of casein serves as an index to the quality or suitability of that particular batch of casein for a particular application. Dolby (1961), working with lactic acid casein, found that casein at a concentration of 9% or less did not give viscosity readings that were a reliable indication of all the properties of the casein. He modified a method using borax so that the casein concentration was made to 15% in borax. This responded to changes in processing variables with a wide range in resulting viscosity. By this method he was able to show that curd that had been coagulated at pH 5.1 yielded casein of high ash content and very high viscosity; coagulation at pH 4.6 produced a curd with a much lower ash content and a better than fivefold reduction in viscosity. High temperatures in washing and drying produced only a minor increase in the viscosity of the casein.

Hayes and Muller (1961) used Dolby's procedure to investigate hydrochloric acid and sulfuric acid caseins, as well as lactic acid casein. They confirmed Dolby's findings that the factors which affect the viscosity of lactic acid casein are the pH of precipitation, the pH at which the viscosity is measured, the concentration of the casein, and the ash content. They further found that these same factors affect the viscosity when the casein is precipitated by hydrochloric or sulfuric acid. At a concentration of 15%, casein in borax has a flat minimum in its viscosity at about pH

7.0 when its calcium concentration is about 1 mg/gm—a good grade of commercial casein. With the addition of calcium the viscosity is still at a minimum at pH 6.5–7.0, but it increases rapidly on either side of this pH range.

The viscosity of caseinate solutions is nonNewtonian (i.e., the measured viscosity depends on the shear rate). Consequently, when one is reporting viscosity of caseinate solutions, the shear rate, temperature, pH, concentration of caseinate, and alkaline additive (borax, NaOH, NH_4OH, etc.) should all be noted. Hayes and Muller (1961) also found that the temperature-viscosity relationship of caseinates is linear when the viscosity is plotted versus the reciprocal of the absolute temperature on a logarithmic scale. However, Hayes *et al.* (1968) found that at concentrations of added calcium above 0.5% the above described relationship departs markedly from linearity.

Food Industry.—According to Poarch (1967) no fewer than 26 food products containing casein or sodium caseinate may be found in the local grocery store. The largest users of caseinates in foods in 1968 were the simulated dairy products (see Chap. 10). These include the coffee whiteners, whipping powders, instant breakfasts, and imitation milks. According to the author cited, it is not inconceivable that in the future, simulated meats such as chicken and ham, and simulated seafoods may be made from spun casein fibers. Caseinates have been found useful as water binders in sausages. This does not mean that casein is used to increase the water content of meat, but rather that it holds the moisture present and thus keeps the meat from drying out.

Hydrolysates.—Historically, soya sauce was the first protein hydrolysate. It was made for centuries in the Orient by cooking together a blend of soybeans and wheat. The cooked mixture was allowed to become moldy, after which it was salted, placed in crocks, and allowed to stand for about six months. In modern terms, enzymes from the blue mold hydrolyzed the proteins of the soybeans and wheat.

The fundamental flavor characteristic of a protein hydrolysate suggests meat. Therefore hydrolysates are used by packers, canners, and other manufacturers who wish either to accentuate or suggest the flavors of meat. In precooked, cured, stored, or comminuted meats much of the flavor is lost. Also dehydrated meats and dry soup preparations are quite low in flavor. Baked items such as savory crackers and snack items are heavy users of protein hydrolysates.

For food purposes hydrolysates are commonly prepared by acid hydrolysis. The proteins commonly used are soy protein, corn protein (zein), gluten, casein, and yeast. The hydrolysate of each protein has a distinct flavor characteristic. Different proteins may be blended to give a specific

flavor characteristic. According to Connell (1966) lighter, more delicately flavored hydrolysates are derived from casein, corn, and rice proteins. These are preferred for fish, pork, and poultry. Darker, heartier hydrolysates are derived from the proteins of soy, yeast, and gluten. These are preferred for beef and mutton.

The hydrolysis is carried out in glass-lined vessels in 10-20% hydrochloric acid. At atmospheric pressure, 10-12 hr are required to complete the hydrolysis. The hydrochloric acid-water solution forms a constant boiling mixture at about 235°F. Under pressure, the hydrolysis can be completed in 4-5 hr. Further details of the acid hydrolysis of casein are disclosed in the patent issued to Hoglan and Kodras (1961).

Enzymatic hydrolysis results in a product free of salt. In acid hydrolysis NaCl is formed in neutralization of the hydrolyzing acid with NaOH. According to Connell (1966) enzyme hydrolysates are usually characterized by off-flavors. Depending on the particular enzymes used, the protein may be partially hydrolyzed to peptides rather than completely to the constituent amino acids. A patent on the enzymatic hydrolysis of casein with trypsin has been issued to Oberg *et al.* (1951). Since this enzyme hydrolyzes the protein chain at the lysine and arginine residues only, the product must consist largely of peptides of varying size. The product is intended for use with syrups and sugars in candy manufacture. A possible factor to be considered in the use of enzymatic hydrolysates of caseins is the finding by Baker and Bertok (1963) that those peptides of casein which form stable foams tend to be antigenic.

Classification of Casein

A classification of edible casein by grade has been established (Federal Register 1968) in the United States. Specifications for the 2 grades are itemized in Table 11.5. In addition to the above compositional analyses, both grades require that the product be free from offensive flavors and

TABLE 11.5

STANDARDS FOR CASEIN BY GRADE

	Extra Grade	Standard Grade
Moisture (max)	10%	12%
Fat (max)	1.5%	2%
Free acid (max)[1]	0.20 ml	0.27 ml
Ash (max)	2.2%	2.2%
Protein content dry basis[2]	95%	90%
Plate count/gm (max)	30,000	100,000
Coliform count (max)/0.1 gm	0	2

SOURCE: Federal Register (1968).
[1] 0.1 *N* NaOH per gm casein.
[2] Protein content = % nitrogen × 6.38.

odors such as sour or cheesy; in physical appearance, both must be white-to cream-colored.

Australia has standards for both acid casein and rennet casein. Acid casein is classified as Special Grade and Standard Grade, and the rennet casein as Grade 1 and Grade 2. Their specifications for Standard Grade Acid Casein are quite similar to the specifications for U.S. Standard Grade.

The classification of edible casein is based on the results of analyses made according to "Methods of Laboratory Analyses for Edible Dry Casein (Acid)" (Gaarbe 1968).

WHEY PROTEINS

About 22 billion pounds of cheese whey was produced in the United States in 1968. The whey contained roughly 2/3 of the total solids of skim milk, but only 1/5 of the protein. An average figure for the protein content of whey would be near 0.7%. This amounts to 154 million pounds of protein. However, the fractionation of whey proteins from whey is expensive because of their low concentration in the whey. When the whey has been concentrated to 55% solids, there will be, on the basis of 0.7% protein originally, only about 5.9% protein in the concentrate. The vacuum concentration and the heat denaturation of whey proteins require the expenditure of power and skilled labor and therefore are expensive operations.

If whey protein is to be removed by heat it is probably best done before concentration as in clarification for lactose manufacture. It can also be done from the concentrate, but the heat precipitated protein is high in lactose and salts and the heavy precipitate is hard to separate from the mother liquor.

The following uses for whey proteins separated by the different processes have been suggested in patent applications: as a component in bakery goods, baby foods, dry pudding mixes, ice cream mixes, salad dressings, whipping agents, and emulsifying agents for toppings. For a hydrolysate of whey proteins, the following uses were suggested: addition to soups, bouillons, fruit juices, condiments, and dietetic preparations.

Methods of Whey Protein Separation

The large number of patents issued on the subject indicates that there are many ways of recovering the whey proteins from the other whey constituents. Some of the newer methods provide a basis for recovering undenatured protein.

Heat Denaturation.—This procedure involves acid precipitation of heat-denatured whey proteins. Patents have been issued for processes in

which the heat denaturation was done with the pH adjusted to neutrality followed by adjustment of the pH to 4.8–5.3, and also for procedures in which the pH was adjusted to 4.7 before heat denaturation. The following is a summary of a procedure described by Burkey and Walter (1947):

(1) With the whey in the vat in which it is to be heated, adjust the acidity if necessary. Whey that has a pH of 6.3 to 6.5 (acidity of 0.11–0.13%) appears to be most stable to heat. Adjustment of the whey to this range of acidity before heat is applied largely prevents premature precipitation of the proteins during the heating period.

(2) Heat the whey to 205° F and stir constantly while the whey is being heated. More recently Guy *et al.* (1967) found that heating to 195° F for 30 min denatured only 80% of the whey proteins. Maximum heat stability of the whey proteins was found in whey containing 20% total solids.

(3) As soon as the whey is heated, the precipitating agent is added with constant stirring until precipitation is complete. About 1 part of concentrated hydrochloric acid per 3000 parts of whey is required. If calcium chloride is the precipitant, 1.3 lb dissolved in water is required for 1000 lb of whey. For complete precipitation by acid, the pH of the hot whey should be between 4.8 and 5.3. When calcium chloride is used as the precipitating agent, the pH for complete precipitation should be between 5.5 and 5.8. As soon as precipitation is complete, stirring is discontinued to allow the precipitated protein to collect.

(4) The precipitated protein may be recovered from the whey by filtering with a Swiss-cheese dipping cloth, by draining off the whey, or by centrifuging.

The whey-protein curd, after cooling and draining, has an acidity between pH 5.6 and 6.0. It contains about 77% moisture, 16% protein, 3.5% lactose, 2.5% fat, and 1.0% ash.

Other Procedures.—The recovery of protein from whey by older methods usually produced a heat denatured product of good nutritive value but with no innate property that would make it of special value for food use. If inexpensive undenatured whey protein preparations were commercially available many new food applications might develop. Thus, commercial fractionation and purification processes are needed that will preserve the natural unique properties of the original protein. The properties of high foaming, whippability, heat coagulation under controlled conditions and an unusual capacity to absorb large quantities of water should be preserved for full realization of whey protein potential. The processes described below are a start in this direction. Gel filtration, electrodialysis, and reverse osmosis are in early stages of commercial development.

Ferric Salt Precipitation.—In one procedure (Block and Bolling 1955), a chelated protein complex is prepared from whey by adding to it a dilute solution of a ferric salt. The ferric ion may be later removed from the product by treating with SO_2. Several patented variations of the use of ferric salts to precipitate the whey proteins exist.

Polymeric Phosphates.—Gordon (1945) precipitated the whey proteins in essentially undenatured form by an original application of a polymeric phosphate procedure. Zboralski (1957) used polymeric phosphates, sodium metaphosphate, or sodium polyphosphate to precipitate the whey proteins after they had been concentrated and heated. Hartman and Swanson (1966) adjusted the pH of cheese whey to about 2.5 followed by addition of 0.5% sodium hexametaphosphate. The whey was not heated. The precipitated proteins gave electrophoretic patterns similar to the native whey proteins.

Anionic Polyelectrolyte.—By this process (Rogers and Palmer 1966) the whey is adjusted to pH 4.0-5.2, preheated to 149°-176° F, heated at 203°-212° F with turbulent mixing to precipitate the protein, held with agitation 0.5-2 min at 203°-212° F, and then mixed with 50 ppm (based on liquid whey) of 1-carbo-methoxy-2-carboxy-3-acetoxybutylene copolymer (molecular weight 200,000) and held with agitation for 2 min. The flocculated sediment may be separated from the whey by screening.

Gel Filtration.—Another procedure for the separation of the whey proteins from their attendant salts and lactose is by gel filtration. This procedure has been applied to skim milk on a pilot plant scale by Samuelsson *et al.* (1967). The application of gel filtration to the fractionation of whey on a laboratory scale is described by Morr *et al.* (1968). Steiner (1968) describes a commercial plant in Sweden which produces a dried protein by preconcentration of whey, lactose separation (optional), gel filtration, final concentration, and spray drying to yield a powder containing 75% protein. These groups of investigators used G-25 Sephadex (Pharmacia Fine Chemicals 1969) as their filtration media. Swanson and Ziemba (1967) describe a gel filtration process on a large scale using cross-linked zeolites for the partial separation of whey proteins from the other whey constituents. Several fractions ranging up to 75% protein were reportedly separated. Further word from Swanson and Associates (Attebery 1969) describes a representative product produced on a pilot scale with a composition: protein 60%, lactose 15%, ash 15%, other 10%. This material is said to produce a meringue-type whip in a solution containing 20% as much protein as egg white.

Electrodialysis of Whey.—For some purposes a partial separation of the whey constituents is more useful than a complete separation would

be. This has been found to be so in the preparation of infant's formula. By means of electrodialysis of both whey and skim milk, the amount of ash in these products is substantially reduced without much decrease in the amount of lactose and protein present in each. By combining electrodialyzed whey solids with electrodialyzed skim milk solids plus some added lactose and fat, a simulated milk can be prepared which has the same composition of whey protein, casein, lactose, fat, and salts as human milk. A description of the preparation of one such infant's formula is that given by Clark et al. (1967).

The first step in fractionation of the whey components is usually removal of part of the lactose by concentrating the whey, crystallizing the lactose and centrifugally removing it. Approximately half the lactose can be readily separated in this way. The remaining concentrate is next treated to remove part of the salts. One process utilizing electrodialysis produces a final product of the following composition: whey protein 28%, salts 10%, and lactose 51% (Anon. 1969).

Reverse Osmosis.—As discussed previously, the separation of whey protein from whey is expensive because of its low concentration. Reverse osmosis, though still very much in the developmental stage, has shown promise as a technique for both concentration and fractionation of whey (McDonough 1968; McDonough and Mattingly 1969; Lowe et al. 1968; Marshall et al. 1968). McDonough and Mattingly (1969) removed over 80% of the water from cottage cheese whey, while retaining over 97% ts. Almost 24% of the salts was removed during the process. The use of more selective membranes, permeable to low molecular-weight salts and sugars, but impermeable to high molecular-weight proteins, should provide a means for producing nearly pure whey protein. By such a system, the whey protein could conceivably be concentrated sufficiently to make subsequent spray drying economically feasible.

Marshall et al. (1968) demonstrated a 2-step procedure for increasing the relative concentration of whey protein in a product by concentrating

TABLE 11.6

COMPOSITION OF WHEY FRACTIONS PREPARED BY REVERSE OSMOSIS FROM A PRODUCT CONTAINING CRYSTALLIZED LACTOSE

Sample	Solids %	Lactose		Protein ($N \times 6.38$)		Other	
		%	% of Solids	%	% of Solids	%	% of Solids
Concentrate	53.3	40.4	75.4	10.5	18.7	2.4	5.9
Supernate	27.9	11.0	39.5	13.1	47.0	3.8	13.5
Infranate	29.7	9.5	32.0	16.9	56.8	3.3	11.2
Precipitate	70.0	63.1	90.0	6.5	9.3	0.4	0.7

SOURCE: Marshall et al. (1968).

to 53% solids, and crystallizing and centrifugally separating the lactose. Table 11.6 gives their data. The 53% concentrate was cooled to 3°C, agitated 24 hr to favor crystallization of lactose, and was then centrifuged 1 hr at 3000 rpm and separated into 3 fractions—a precipitate, infranate, and supernate. The precipitate was about 90% lactose while the infranate contained 57% of its solids as protein.

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